PREreview of bioRxiv article "NRG1-mediated recognition of HopQ1 reveals a link between PAMP and Effector-triggered Immunity"

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Abstract

This is a review of Brendolise et al. bioRxiv 293050; doi: https://doi.org/10.1101/293050 posted on April 1, 2018. This paper adds to a current body of research detailing the resistance mechanism triggered by the *Pseudomonas syringe pv. tomato* effector HopQ1 in the model plant *Nicotiana benthamiana*. This plant can be used as a source of novel disease resistance genes against plant pathogens.

Summary

This paper adds to a current body of research detailing the resistance mechanism triggered by the *Pto* effector HopQ1 in *N. benthamiana*; a 2017 paper identified the cognate resistance gene, Roq1 (Schultink et al., 2017), and a very recent publication showed that the CCR-NB-LRR (RNL) NRG1 is a required downstream component to mediate the response (Qi et al., 2018).

This paper builds on the authors' previously published method to identify candidate R genes in *N. benthamiana*for a given target effector. In this paper, they employ this method to determine the R gene(s) required to confer recognition to the *Pto*effector HopQ1, which "is the sole effector [from *Pto*]... recognized in both *Nb* and*Nicotiana tabacum*" (lines 96- 97). They argue that the widespread conservation of HopQ1 among phytopathogenic bacteria means that, "the identification of R proteins able to recognize [this] effector is potentially valuable to achieve resistance to a wide range of plant pathogens" (lines 106- 107).

The authors show that (1) the RNL NRG1 is required for HopQ1-mediated cell death in *N. benthamiana* and that NRG1 silencing in *N. benthamiana* compromises the resistance to DC3000. They also show evidence that (2) expression of NRG1 within the normally susceptible plant *Arabidopsis thaliana* is sufficient to confer HopQ1-triggered cell death, as well as confer resistance to DC3000. In addition, the authors present evidence that (3) NRG1 is induced at an early time-point following PTI activation and hypothesize that this represents a link between PTI and ETI.

The first (1) finding of this paper is convincing, although shown in a previous publication (Qi et al 2018). The results from the second (2) line of experimentation are more preliminary, with open questions stemming from a lack of adequate controls. The final (3) finding of this paper is underdeveloped, and further experimental validation is required to suggest that NRG1 represents, "a link between PAMP and effector-triggered immunity" as stated in the title.

Findings and comments

(1) NRG1 is required for HopQ1 recognition in *N. benthamiana*(Nb) and NRG1 silencing promotes *Pto* proliferation in Nb: Agro-mediated silencing of NRG1 in Nb reduces HopQ1-triggered HR; VIGS-mediated silencing of NRG1 in Nb promotes *Pto*proliferation

o At no point is the resistance gene Roq1 mentioned in these sections, even though it is known that HopQ1 is directly recognized by Roq1 in *N. benthamiana* (Schultink et al 2017). As written, it is not clear how the authors propose that NRG1 functions in mediating the cell death response triggered by Roq1 recognition of HopQ1, or how NRG1 functions in restricting *Pto*proliferation.

o Previous publication reporting similar findings is not referenced (Qi et al 2018)

o Figure 1a-b: It would be more robust to include quantification of reps. An explanation of what hp#26, hp#27, u135, u111 are targeting would be helpful the figure legend.

o There appears to be mis-referencing of figure 1 in this section, e.g. line 232- 233 should refer to fig 1c rather than 1d.

(2) Expression of NRG1 in *A. thaliana*(At) confers recognition to HopQ1 and restricts *Pto*growth: HopQ1 and GFP reporters were transiently expressed in 35s:NbNRG1 transgenic At lines using biolistic delivery, and HR was evaluated by quantifying a reduction of the GFP signal resulting from cell death; found a greater reduction in GFP signal in 3/5 NRG1 lines, compared to GUS lines; in these 3 lines, *Pto*proliferation was also reduced in infection assays

o The robustness of the method used to assay the cell death response is unclear. There is inherent variability in the system as demonstrated by the differences in GFP levels in the EV control, so can it be claimed that HopQ1 has less GFP expression due to the activation of HR? A reduction in GFP over time may serve as a better internal control than a comparative approach.

o As in the previous sections, no mention of Roq1. It's mentioned in the discussion that At may have a functional homolog of Roq1, but that should be stated here and explored in some manner, otherwise these results are difficult to interpret.

o Figure 3. X axis labels are unclear, use clearer names to distinguish transgenic lines.

o The conclusion that NRG1 expression in At induces recognition of HopQ1 to restrict DC3000 growth is not justified from the experiments shown because (1) NRG1 has been implicated in the function of multiple R genes and (2) overexpression can cause HR. Better controls are required to make this claim, such as assaying a DC3000 strain lacking the HopQ1 effector to determine if NRG1 affects other R protein / effector interactions within At.

o Although mentioned in both the introduction and discussion, the authors don't explore whether HopQ1 and XopQ, a homolog from *Xanthomonas*, are recognized by a shared resistance mechanism. Either XopQ should be downplayed in these sections, or the resistance mechanism trigged by this effector should be experimentally explored.

(3) NRG1 expression is induced at the early stages of the bacterial infection: (1) with biolistic delivery system of HopQ1 in Nb, the authors found no reduction of GFP signal (whereas a reduction in At was observed); when leaves were pre-treated with Agro, a significant reduction of GFP signal was measured; (2) when Nb leaves were infiltrated with Flg22 peptide, NRG1 expression was induced compared to NRG2 (N-terminally truncated) control

o The effect of agro could be explained by technical problems with the experiment rather than the activation of PTI, particularly as this result wasn't observed in Arabidopsis.

o It cannot be concluded from these experiments that PTI activation is required for the function of NRG1. Agro infiltration could have multiple effects on the plant, thus more controls are required. For example, is there the same effect with flg22 treatment +/- FLS2?

o The expression analysis lacks proper controls, e.g. unrelated genes not involved in PTI or functional R genes that do not require PTI priming to induce HR. Moreover, the expression levels of NRG1 upon flg22 treatment do not necessarily reflect the relative abundance of this protein within the cell.

Specific comments

- Lines 42-43: "Propose a model based on the dual requirement of a CNL and a TNL that could extend beyond HopQ1 detection..." This model has been proposed previously, and the appropriate publications should be referenced.

- Line 83- 84: "resistance from non-host species comprises various mechanism"; this is quite vague. The idea that non-host resistance could confer more durable resistance could be elaborated on as this appears to be the rationale behind the experimental design.

- Lines 257- 260: "However, the expression of HopQ1 in Arabidopsis does not trigger any HR, suggesting that AtNRG1.1 and AtNRG1.2 are not fully functional homologs of NRG1 and/or able to recognize HopQ1." This seems misleading as it is Roq1, not NRG1, that has been implicated in direct HopQ1 recognition.

- Lines 280- 281: on the At-NRG1 lines, "These results suggest that NRG1 expression in Arabidopsis induces recognition of HopQ1..." 'Induces' takes on a different meaning in this context; use "leads to," which has a more indirect connotation that better fits with the data.

- Line 364- 366: this should be brought up earlier in the manuscript.

- NRG1 is referred to as both a CNL and an RNL, please be consistent with the terminology used.

Reviewers

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References

Tiancong Qi, Alex Schultink, Julie Pham, Myeong-Je Cho, and Brian John Staskawicz. NRG1 is required for the function of the TIR-NLR immune receptors Roq1 and RPP1 in Nicotiana benthamiana. mar 2018. doi: 10.1101/284471. URL https://doi.org/10.1101%2F284471.

A Schultink, T Qi, A Lee, AD Steinbrenner, and B Staskawicz. Roq1 mediates recognition of the Xanthomonas and Pseudomonas effector proteins XopQ and HopQ1. *Plant J*, 92:787–795, Dec 2017.